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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/497, 31/22, C12Q 1/04

A1

(11) International Publication Number: **WO 97/30351**

(43) International Publication Date: 21 August 1997 (21.08.97)

(21) International Application Number: PCT/EP97/00712

(22) International Filing Date: 13 February 1997 (13.02.97)

(30) Priority Data:
9603031.7 14 February 1996 (14.02.96) GB

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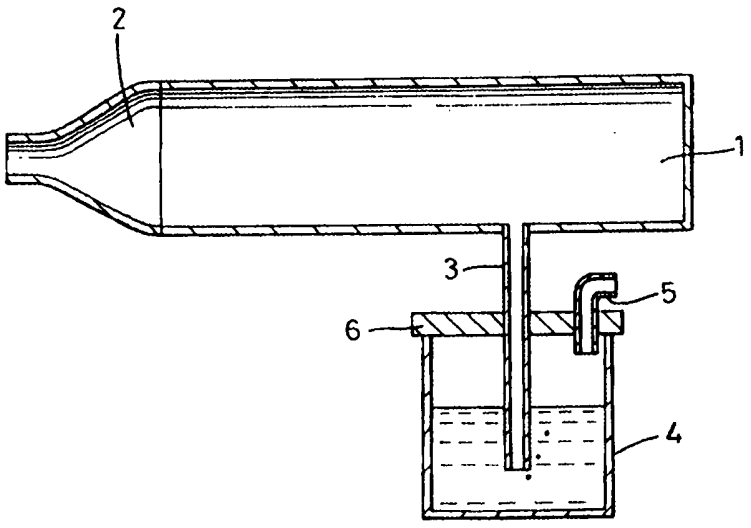
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DIAGNOSTIC METHOD FOR MEASURING THE AMMONIA CONTENT IN BREATH



(57) Abstract

Ammonia in the exhaled breath of human subjects, for example subjects infected with *H. pylori* is detected by capturing the ammonia and contacting the captured ammonia with an indicator system which exhibits a detectable visible change in the presence of ammonia. The ammonia may be captured in an acidic medium. The indicator system may react directly with the ammonia, for example, the indicator system may contain transition metal ions which form coloured complexes with ammonia or it may comprise an acid base indicator. Alternatively, the capturing of the ammonia and the contacting it with the indicator system may be two separate stages. The indicator system in this case may be based on the pyridine-pyrazolone or indophenol reactions.

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DIAGNOSTIC METHOD FOR MEASURING THE AMMONIA CONTENT IN BREATH

This invention relates to the detection of ammonia in the breath of subjects, for example subjects suffering renal failure or *Helicobacter pylori* (*H pylori*) infections in the gastrointestinal tract. *H pylori* infection is believed to be implicated in the development of gastritis, peptic ulceration and gastric cancer. Detection of the presence of *H pylori* infection would therefore enable those patients who are infected and who have, or are at risk of developing, symptoms of dyspesia, gastritis, peptic ulceration and gastric cancer to be identified so that appropriate treatment can be given.

H pylori produces carbon dioxide and ammonia by the action of its urease on urea present in low levels in all body fluids. One standard test to detect the carbon dioxide produced in this reaction involves the administration to the patient of an oral dose of C-isotopically-labelled urea followed by the measurement of the amount of labelled carbon dioxide exhaled in the breath of the patient by mass spectrometry or scintillation counting. This test is expensive and can only be performed under the supervision of medical practitioners where access to a mass spectrometer or scintillation counter is available.

The amount of ammonia produced in the stomach of patients infected with *H pylori* can be measured by taking a sample of the gastric juice by means of endoscopy. This is an invasive technique requiring the presence of skilled medical staff. Immunological tests exist to detect antibodies to *H pylori* in body fluids (eg blood or saliva). These tests can continue to give false positive results long after any *H pylori* infection has been eradicated.

It has been proposed in a paper published after the priority date of the present application that ammonia in the breath of a patient infected with *H pylori* can be measured by means of the so-called selected-ion flow tube (SIFT) approach [Smith D and Spanel P - Rapid Communications in Mass Spectrometry, Vol 10, 1183-1198 (1996)]. At the time of writing the article, this approach did not provide a portable detection device for the ammonia so produced. The approach depends on mass spectrometry, ion chemistry and flow tube principles so even if a portable unit could be developed it would be expensive to use and require the presence of skilled operators.

The present invention provides a non-invasive, speedy and accurate means to detect the ammonia in the exhaled breath of the patient infected with *H pylori*. The test can be conducted by personnel with only a small amount of training or by the patient themselves.

A first aspect of the present invention provides a method of detecting ammonia in the exhaled breath of a patient which method comprises capturing the ammonia present in the exhaled breath and contacting said captured ammonia with an indicator system which exhibits a detectable visible change in the presence of ammonia.

A second aspect of the present invention provides a detection device for detecting ammonia in the exhaled breath of a patient, which device comprises a chamber in which are located means to capture the ammonia in the breath and means whereby the ammonia so captured can be contacted with an indicator system which exhibits a detectable change in the presence of ammonia and means whereby the exhaled breath of the patient is directed into the chamber.

The ammonia may be captured by an indicator system which reacts directly with the ammonia to give a detectable visible change. Preferably the

indicator system is incorporated into or is absorbed or coated onto a support. The support may be porous and may comprise a fibrous material or a particulate material which may be sintered or in the form of beads. Suitable materials include porous cellulose materials (eg paper), nylon, nitrocellulose, zeolites, sintered solid materials such as calcium phosphate or a support in the form of beads for example of agarose or carageenan beads. The indicator system may be incorporated into the porous support so that it is present uniformly throughout the support or it may be absorbed or coated onto the outer surfaces of the support. In the case of an indicator system uniformly dispersed through the porous support the indicator system will be incorporated into the support during the manufacturing process but in the case of an indicator which is absorbed or coated onto the surface the indicator will be absorbed or coated onto a preformed support which may be the interior surface of the tube into which the breath to be tested is exhaled.

The support should have a large surface area to maximise the contact between the exhaled ammonia and the indicator to ensure maximum sensitivity. The indicator system may provide a qualitative test which indicates merely the presence or absence of ammonia in the exhaled breath or it may provide a quantitative test in which the amount of ammonia in the exhaled breath can be determined. Such a quantitative test may rely on an increase in the intensity of the colour change exhibited by the indicator system in the presence of increased amounts of ammonia to provide a determination of the amount of ammonia present. Alternatively an indicator system may be used which indicates the amount of ammonia present by the proportion of the support over which the indicated change has occurred in the presence of ammonia.

The indicator system must be one which detects only ammonia and is not influenced by the presence of any other compound which might be found in the exhaled breath of the patient so as to exclude the possibility of false

positives. Preferably the indicator system should be one which undergoes a distinct colour change in the presence of ammonia so that a positive result can be determined by visual inspection only. However, other means, for example electronic detectors, may be used to determine that the change indicating the presence of ammonia has occurred. The change produced must be stable for a sufficient period of time for the user to be able to detect the change and thus become aware of a positive test. The longer the change remains apparent the more likely it is for the user to notice the change. The change should preferably be stable for at least one minute and preferably at least 5 minutes. Preferably the change occurs immediately the exhaled ammonia comes into contact with the indicator. If there is a period of time after contact with the exhaled air during which the detectable change in the indicator develops, then the development must be uniform and consistent so that the user can be provided with detailed instructions on how to undertake the test. If the amount of ammonia is to be determined quantitatively as a result of a colour change which becomes more intensive in the presence of larger amounts of ammonia then the user may be provided with a shade chart against which to compare the test result or the intensity of the colour may be measured with instruments eg a spectrophotometer.

The indicator may contain one or more first or second series transition metal ions from Groups IB, VIIB or VIIIB of the Periodic Table which indicate the presence of ammonia by means of complex formation between the transition metal ion and the exhaled ammonia. Complexes of transition metal ions can be highly coloured and can therefore form the basis of a direct indicator of the presence of ammonia. In the case of transition metal ions which can exist in more than one valency or oxidation state the ammonia may form a complex with the metal ion in one valency or oxidation state and that complex may be more susceptible to reduction or oxidation to another valency or oxidation state. If the colour exhibited by the transition metal ion complex in the second valency or oxidation state differs from that of the original complex,

a detectable colour change will be seen which indicates the presence of ammonia in the exhaled breath sample. Suitable transition metal ions would include nickel [Ni(II)], cobaltous [Co(II)], ferrous [Fe(II)], cuprous [Cu(II)] and manganous [Mn(II)]. The transition metal ions may be in the form of simple salts with counterions such as chloride or sulphate ions. The salts should be soluble so that a solution can be used to impregnate or coat the indicator support. The suitability of transition metal salts to be used as indicators can be demonstrated as follows. An aqueous ammonia solution was diluted until the concentration of ammonia was in the micromolar range. A fibrous support which had been immersed in an aqueous solution of the transition metal salt was then held above the diluted aqueous ammonia. It was found that ferrous chloride and cobaltous chloride gave a colouration which developed in 2 to 4 minutes and achieved its maximum intensity after about 10 minutes. Observable colour changes were also detected with manganous chloride, cuprous chloride, cuprous sulphate and nickel chloride. Mixtures of transition metal ions may also be used.

The indicator system may comprise an acid/base indicator which is one colour at acid or neutral pH values but which changes to a different colour at higher pH values. Ammonia is believed to be the only basic gas present in breath which would cause such an indicator to change colour. The acid/base indicator may be incorporated into or absorbed or coated onto a porous support of the type described hereinbefore. Suitable acid/base indicators include 4-nitrophenol, bromothymol blue and bromocresol purple.

The present invention may be performed in two distinct stages. In the first the ammonia is captured and then measured or detected by the indicator system in the second stage. For example the breath of the patient may be passed through an acidic medium into which the ammonia is absorbed by salt formation. The acidic medium can then be contacted with an indicator such as the transition metal ions listed above to provide a visible indication of the

presence of ammonia. If the acidic capture conditions are not the most appropriate for the formation of the transition metal complexes then the pH of the system can be adjusted to give more favourable conditions for complex formation. The acidic medium may be a dilute aqueous solution of an acid, for example hydrochloric, sulphuric, nitric or phosphoric acid or an organic acid, for example acetic, citric, tartaric or succinic acids or tungstic acid. The acid may be absorbed or coated on to a porous support which may be a fibrous material eg paper, absorbent cellulose fibres, nylon or nitrocellulose or may be a particulate material eg zeolites, sintered solids or in the form of beads. Alternatively the acid may be absorbed onto a porous coating applied to a supporting member. For example, the acid may be absorbed onto a material which is coated onto the walls of the chamber in which the ammonia is captured or onto solid supports eg plates or beads contained in the chamber. It may be necessary to add water to the acid immediately prior to use to ensure that the acid efficiently captures the ammonia in the breath. Any water added must be purified to ensure that it does not contain any ammonia or other materials which could give rise to false results.

The captured ammonia may be detected by utilising the ammonia as a reactant in one stage of a multistage reaction which gives rise to a coloured end point. Examples of suitable multistage reactions include the pyridine-pyrazolone reaction and the indophenol reaction both of which give a coloured material as their end point.

The pyridine-pyrazolone reaction has been described by Okita and Kanamori [Atmospheric Environment, Vol 5, 621-627 (1971)] and by Kruse and Mellon [Analytical Chemistry, Vol 25, 1188-1192 (1953)]. In this test the aqueous sample is buffered to pH 3.7 with a sodium acetate/acetic acid buffer and then chloramine-T is added. After a delay of 90 seconds a pyridine-pyrazolone reagent is added and a purple colour develops if ammonia had been present in the sample. The purple coloured species is soluble in

tetrachloromethane and can be extracted into this solvent for quantitative analysis.

The indophenol reaction (also known as the Berthelot reaction) has been discussed by Liddicoat, Tibbitts and Butler (Limnol. Oceanogr., Vol 20, 131-132 (1975)], by Patton and Crouch [Analytical Chemistry, Vol 49, 464-469 (1977)] and by Ngo, Phan, Yam and Lenhoff [Anal. Chem., Vol. 54, 46-49 (1982)]. The method involves the reaction of ammonia with hypochlorite ions to form monochloramine. The chloramine then reacts with a phenolate anion in the presence of a catalyst and hydroxyl ions to give quinone chlorimide which then reacts with phenolate ion to give indophenol which is blue and the production of this blue colouration is indicative of the presence of ammonia in the breath sample.

The hypochlorite ions may be supplied by a solution of sodium hypochlorite in aqueous sodium hydroxide solution. Such solutions are available commercially but are known to be unstable. Solids which give hypochlorite ions on solution with water are known. An example of a suitable material is sodium dichloroisocyanurate. The phenolate anion may be derived from phenol itself or may be derived from any substituted phenol which is capable of undergoing the reaction described above to give a coloured substituted indophenol. Suitable substituted phenols include 2-chlorophenol, 2-methyl- phenol (o-cresol) and 2,6-dimethylphenol. Suitable catalysts include sodium nitroprusside, potassium ferrocyanide, manganous ions or acetone. At ambient temperatures the formation of the coloured indophenol is slow and it takes approximately one hour for the colour to develop fully. If the reagents are placed in an oven at 37°C then the colour develops in a quarter of an hour whereas if the reagents are heated in a microwave oven to just below boiling point the colour develops in a matter of seconds.

The invention will be illustrated by the following non-limiting description of detection devices according to the present invention. The description has reference to the accompanying drawings in which:-

Figure 1 is a schematic cross-sectional representation of a first embodiment of the invention.

Figure 2 is a schematic cross-sectional representation of a second embodiment of the invention.

Figure 3 is a schematic cross-sectional representation of a third embodiment of the invention.

Figure 4 is a schematic cross-sectional representation of the embodiment shown in Figure 3 during the analysis phase.

Figure 5 is a schematic cross-sectional representation of a fourth embodiment of the invention.

Figure 1 shows a detection device having a tube 1 closed at one end having a mouthpiece 2 which may incorporate a non-return valve (not shown) and which may be integrally formed with the tube I or may be removably attached to it. A side tube 3 passes from the interior of the tube 1 to the interior of a collection chamber 4. The collection chamber 4 has an exhaust tube 5 which is either open to the atmosphere or is attached to gas-volume measuring means (not shown). The collection chamber is closed by a cover 6. The side tube 3 may be provided with coupling means (not shown) to enable the collection chamber 4 together with the cover 6 and the tubes 3, 5 to be separated from the tube 1. In use the subject exhales through the mouthpiece into the tube 1 and the exhaled breath passes through the side tube 3 into the collection chamber 4 which contains a medium which captures the ammonia in

the breath. The medium may be a solution of a weak acid (eg phosphoric acid) or may be a support (eg beads or a gel) coated with an acidic ammonia-absorbing reagent. The exhaled breath which has been scrubbed of its ammonia passes out of the chamber 4 through the exhaust tube 5. The exhaust tube 5 may be attached to a balloon which is inflated as the subject exhales. When the balloon is fully inflated a predetermined volume of exhaled breath will have passed through the collection chamber. Alternatively, the predetermined volume of exhaled breath may be determined by means of a piston which moves within a tube by a set distance or the breath may be split between the tube 1 and a second tube (not shown) containing a measuring system which reacts to the presence of carbon dioxide or moisture in the exhaled breath (eg cobaltous chloride impregated onto silica gel). After the ammonia has been captured the ammonia may be detected by any of the methods described above. For example, solutions of phenol and alkaline hypochlorite may be added to initiate the indophenol reaction. The detection of the ammonia may be performed immediately or the chamber 4 may be sealed and its contents assayed later.

Figure 2 shows a second embodiment of the present invention comprising a tube 11 containing an absorbent porous mass (shown schematically as 12) which is treated with an acidic medium into which the ammonia from the exhaled breath of a subject is captured. Before use the tube 11 is sealed by end caps 13, 14 which engage the open ends of the tube 11. Alternatively the ends of the tube may be sealed prior to use by ruptureable membranes (not shown). The porous mass 12 may act as a support for a liquid acidic medium coated or absorbed thereon. The caps 13, 14 will prevent the porous mass drying out prior to use. Alternatively the porous mass may support a solid acidic material which is activated immediately prior to use by the addition of a measured amount of water to the tube 11. In this case the caps 13, 14 sealing the tube 11 will prevent premature activation by atmospheric moisture.

To use the device containing a coated or absorbed liquid acidic medium both end caps 13, 14 are removed, a mouthpiece 15 is fitted and the patient exhales through the tube 11.

To use the device in which the acidic medium on the porous mass 12 is a solid the end cap 13 is removed and a specified volume of water added. The cap 13 is replaced and the tube shaken until the porous mass has taken up the water activating the previously dry acidic medium. Both end caps 13, 14 are then removed and the mouthpiece 15 fitted to enable the subject to exhale through the tube 11.

Means such as those described above may be attached to the end of the tube 11 remote from the mouthpiece to ensure that a predetermined volume of exhaled air passes through the porous mass 12. The tube may then be sealed and the assay of the ammonia captured in it may be performed later. Alternatively one end of the tube may be reclosed by replacing the cap on it and the remaining parts of the indicator system may be added for immediate detection of the ammonia. For example, solutions of phenol, alkaline hypochlorite and catalyst may be added to initiate the indophenol reaction.

In an alternative embodiment of the invention shown in Figure 2 the porous mass 12 contains a solid acid and the indicator eg a transition metal indicator system as described above in anhydrous form. The acid and indicator are activated immediately prior to use by the addition of water.

In a further embodiment of the invention shown in Figure 2 which utilises the indophenol reaction, the porous mass 12 contains a dry acid and the components of the indicator system (the phenol, a solid source of hypochlorite ion eg sodium dichloroisocyanurate and the catalyst eg

potassium ferrocyanide) encapsulated in a water-soluble coating which dissolves slowly in the presence of water to release the components. The components may be encapsulated separately or may be encapsulated together if the mixed components in the dry state would have a satisfactory shelf-life prior to use. Immediately prior to use a specified amount of water is added which immediately activates the acidic capture medium and slowly releases the reagents for the indophenol reaction. The ammonia in the subject's exhaled breath will then be captured by the immediately-activated acidic medium and subsequently the components needed to initiate the indophenol reaction will become available. The water needed to activate the device may be added from an external source or may be contained within the device but kept separate from the porous mass 12 prior to use. The water may be contained in a closed rupturable container held within the tube 11 (not shown).

The embodiment shown in Figures 3 and 4 comprise a tube 21 which is open at both ends and which contains a porous mass 22 in which is located an acidic medium to absorb the ammonia and at least part of the indicator system. A mouthpiece 23 can be fitted to enable the subject to exhale into the tube. The end of the tube remote from the mouthpiece is fitted with a porous plug 23. After the patient has exhaled through the tube the mouthpiece is removed and the tube 21 is stood in a base 24 which seals the bottom of the tube 21 to prevent the egress of liquid. A container 25 contains a solution of the remaining parts of the indicator system. The container 25 has an opening in one wall thereof into which the end of the tube 21 containing the porous plug 23 may be inserted. When the tube is inserted into the opening, resilient seals 26 around the opening grip the outside of the tube 21. Prior to use the opening is sealed by a membrane 27 to prevent the egress of liquid. However, as the container 25 is pushed on to the end of the tube 21 as shown in Figure 4, the end of the tube is pushed against the membrane 27 causing it to rupture to release the contents of the container 25 into the tube

21. The liquid contents of the container 25 pass through the porous plug 23 and contact the materials inside the porous mass 22. If the indophenol reaction is being used to detect the presence of ammonia, the porous mass 23 may contain the phenol and the catalyst and the container 25 may contain an aqueous solution of alkaline hypochlorite. As the alkaline hypochlorite enters the porous mass it neutralises the acid and dissolves the phenol and catalyst which are required to initiate the indophenol reaction. The ends of the tube 21 should be shaped so as to prevent the inadvertent fitting of the container 25 or the base 24 to the wrong end of the tube. To initiate the indophenol reaction the tube in its holder with the container 25 attached may be placed in a microwave oven until the reaction is complete and a blue colour will be evident in the porous mass 22 if ammonia was present in the breath of the subject.

Figure 5 shows a further embodiment of the present invention which comprises a tube 31 containing a porous filling 32 onto which an acidic ammonia-absorbing reagent and a part of the indicator system are absorbed or coated. The outer surface of the tube 31 has a plurality of radial ribs 33. One end of the tube is closed by a non-return valve 34. This one end of the tube may be formed into the shape of a mouthpiece or may be adapted to interengage with a separate mouthpiece (not shown). The one end of the tube is surrounded by a hemispherical sleeve 35 the outer edge 36 of which extends at least as far as the one end of the tube. Prior to use the sleeve 35 may be sealed by means of a removeable film (not shown) sealed to its outer edge 36. The other end of the tube carries a cap 37. The cap 37 is rotatable from a first position to a second position. The outer surface of the cap 37 is provided with radial grooves (not shown) which are not aligned with the ribs 33 in the first position but which are aligned with the ribs 33 in the second position enabling the cap 37 to be moved towards the mouthpiece end of the tube 31. The cap encloses a chamber 38 which when the cap is in its first position is sealed with a foil 39. The chamber 38 contains the remaining part of the indicator system in liquid form. The cap has one or more outlet apertures 40

through which the exhaled air which has passed along the tube 31 is vented to the atmosphere. Prior to use the outlet apertures 40 may be sealed by a removeable seal. An inner piercing tube 41 extends from the end of the tube 31 remote from the mouthpiece towards the foil 39 sealing the chamber 38. The inner end of the piercing tube is supported coaxially within the end of the tube 31 by an apertured plate (not shown) or radial ribs (not shown) joining the interior wall of the tube 31 to the outer wall of the piercing tube 41. The apertures or the space between the ribs must have a sufficiently large area that the flow of exhaled air is not unduly hindered. The outer end of the piercing tube 41 may be shaped to ensure that the tube can pierce the seal 39. In use, the seal over the outlet apertures 40 and, if present the seal on the outer end of the sleeve 35, is removed. The subject then exhales through the tube 31. The ammonia is captured by the ammonia-absorbing reagent in the filling 32 and the exhaled breath passes through the aperture(s) 40. The device is then stood upright on the outer edge 36 of the sleeve 35. The cap 37 is then rotated until the ribs 33 on the tube 31 are aligned with the grooves on the cap 37. The cap 37 is then pushed towards the mouthpiece. The grooves move over the ribs 33. As the cap is pushed the outer end of the piercing tube 41 pierces the seal 39 and the contents of the chamber flow into the interior of the tube 31 through the piercing tube 41.

If the indicator system is based on the indophenol reaction, the phenol and the catalyst may be absorbed or coated onto the filling 32 and the liquid in the chamber 38 may be an alkaline solution of hypochlorite .

In use, the test may be conducted on patients first thing in the morning before they have ingested any food or drink which might contain sources of ammonia or urease. Foods which contain sources of ammonia or ureases which could mimic *H pylori* urease (for example pineapple), would have to be avoided by the patient. If the amount of urea present in the gastric environment is insufficient the patient could be predosed with a known amount

of urea or other suitable material to provide more substrate on which the *H pylori* urease can act resulting in more detectable ammonia in the exhaled breath of the patient. The urea or other substrate for the *H pylori* urease may be given in a capsule or a coated tablet to minimise dry interaction with urease-containing bacterial plaque in the oral cavity. In the event that the patient is predosed, there will need to be a period of time (for example 30 minutes) after ingestion of the urea before the amount of ammonia in the exhaled breath is detected.

It is known that ureases may be present in the oral cavity and these, if present, could react with urea to give ammonia. It may therefore be preferable for the patient to cleanse the oral cavity by the use of a toothbrush or mouthwash before the test is administered. Any dentifrice or mouthwash used must not contain any ingredients which might interfere with the test.

The ability of the device and method of the present invention to detect the presence of ammonia in breath was demonstrated by the following experiment using the indophenol reaction as the colorimetric indicator.

A solution (Solution A) of phenol (2.50 g) and potassium ferrocyanide (0.5 g) in water (total volume = 250 ml) and a solution (Solution B) of commercially available sodium hypochlorite solution (4-20% available chlorine - 4.2 ml) and sodium hydroxide (2.50 g) in water (total volume = 500 ml) were made up.

The subject was asked to exhale into a 100 ml glass syringe. The piston of the syringe was forced beyond the 100 ml mark. The piston was then pushed back to the 100 ml mark and 0.04N phosphoric acid (5 ml) was injected into the syringe. The syringe was then sealed, shaken and stood at ambient temperature for 90 minutes. The contents of the syringe were transferred to a 25 ml conical flask and 5 ml of each of Solution A and Solution

B were added. The mixture was placed in a microwave oven and heated on full power for 35 seconds. If the breath sample contained ammonia then a blue colouration develops. The intensity of the colour is dependent on the amount of ammonia and a quantitative determination of the amount can be obtained spectrophotometrically at 635 nm.

Subjects who are known to be infected with *H pylori* give a more intense blue colour than subjects who are known to be uninfected. The test is therefore capable of distinguishing subjects who are infected with *H pylori* from those who are not. This would enable it to be used to screen patients for the presence of infection or to check that the prescribed drug regime had resulted in the eradication of the infection. Unlike immunological tests, the test according to the present invention can be used soon after the drug treatment as, in the absence of *H pylori* infection, no ammonia will be generated from urea in the stomach. In practice, however, a delay of one month after drug treatment would be more usual. If the tests described above showed the presence of ammonia in the exhaled breath of the patient, the patient may be prescribed a drug regime to eliminate the infection or may be referred for further medical diagnosis by, for example, more detailed instrumental analysis of breath (eg a standard test such as the C-13 labelled urea breath test) or body fluids (eg an immunological assay) or endoscopy.

CLAIMS

1. A method of detecting ammonia in the exhaled breath of a patient which method comprises capturing the ammonia present in the exhaled breath and contacting said captured ammonia with an indicator system which exhibits a detectable visible change in the presence of ammonia.

2. A method as claimed in claim 1 in which the ammonia is captured by an indicator system which reacts directly with the ammonia to give the detectable visible change.

3. A method as claimed in claim 2 in which the indicator system comprises a first or second series transition metal ions from Groups IB, VIIB and VIIIB of the Periodic Table which indicates the presence of ammonia by the formation of coloured complexes of the metal ion with ammonia.

4. A method as claimed in claim 3 in which the transition metal ion is nickel, cobaltous, ferrous, cuprous or manganous.

5. A method as claimed in claim 2 in which the indicator system is an acid/base indicator.

6. A method as claimed in claim 5 in which the acid/base indicator is 4-nitrophenol, bromothymol blue or bromocresol purple.

7. A method as claimed in claim 1 in which the ammonia is captured in a first stage and is then measured or detected by the indicator system in a second stage.

8. A method as claimed in claim 7 in which the ammonia is captured by salt formation in an acidic medium.

9. A method as claimed in claim 8 in which the acidic medium is a dilute aqueous solution of an acid or a solid acid.

10. A method as claimed in claim 9 wherein the dilute aqueous acid is hydrochloric, sulphuric, nitric or phosphoric or acetic acid.

11. A method as claimed in claim 9 in which the acid is acetic, citric, tartaric, succinic or tungstic acid.

12. A method as claimed in any one of claims 8 to 11 in which the acidic medium is absorbed or coated onto a porous support or a porous coating applied to a supporting member.

13. A method as claimed in claim 7 in which the indicator system is based on the pyridine-pyrazolone reaction or the indophenol reaction.

14. A detection device for detecting ammonia in the exhaled breath of a patient, which device comprises a chamber in which is located means to capture the ammonia in the breath and means whereby the ammonia so captured can be contacted with an indicator system which exhibits a detectable change in the presence of ammonia and means whereby the exhaled breath of the patient is directed into the chamber.

15. A device as claimed in claim 14 in which the ammonia is captured by an indicator system which reacts directly with the ammonia to give the detectable visible change.

16. A device as claimed in claim 15 in which the indicator system comprises a first or second series transition metal ions from Groups IB, VIIB and VIIIB of the Periodic Table which indicates the presence of ammonia by the formation of colour complexes of the metal ion with ammonia.

17. A device as claimed in claim 16 in which the transition metal ion is nickel, cobaltous, ferrous, cuprous or manganous.

18. A device as claimed in claim 15 in which the indicator system is an acid/base indicator.

19. A device as claimed in claim 18 in which the acid/base indicator is 4-nitrophenol, bromothymol blue or bromocresol purple.

20. A device as claimed in claim 14 in which the ammonia is captured in a first stage and is then measured or detected by the indicator system in a second stage.

21. A device as claimed in claim 20 in which the ammonia is captured by salt formation in an acidic medium.

22. A device as claimed in claim 21 in which the acidic medium is a dilute aqueous solution of an acid or a solid acid.

23. A device as claimed in claim 22 wherein the dilute aqueous acid is hydrochloric, sulphuric, nitric or phosphoric or acetic acid.

24. A device as claimed in claim 22 in which the acid is acetic, citric, tartaric, succinic or tungstic acid.

25. A device as claimed in any one of claims 21 to 24 in which the acidic medium is absorbed or coated onto a porous support or a porous coating applied to a supporting member.

26. A device as claimed in claim 20 in which the indicator system is based on the pyridine-pyrazolone reaction or the indophenol reaction.

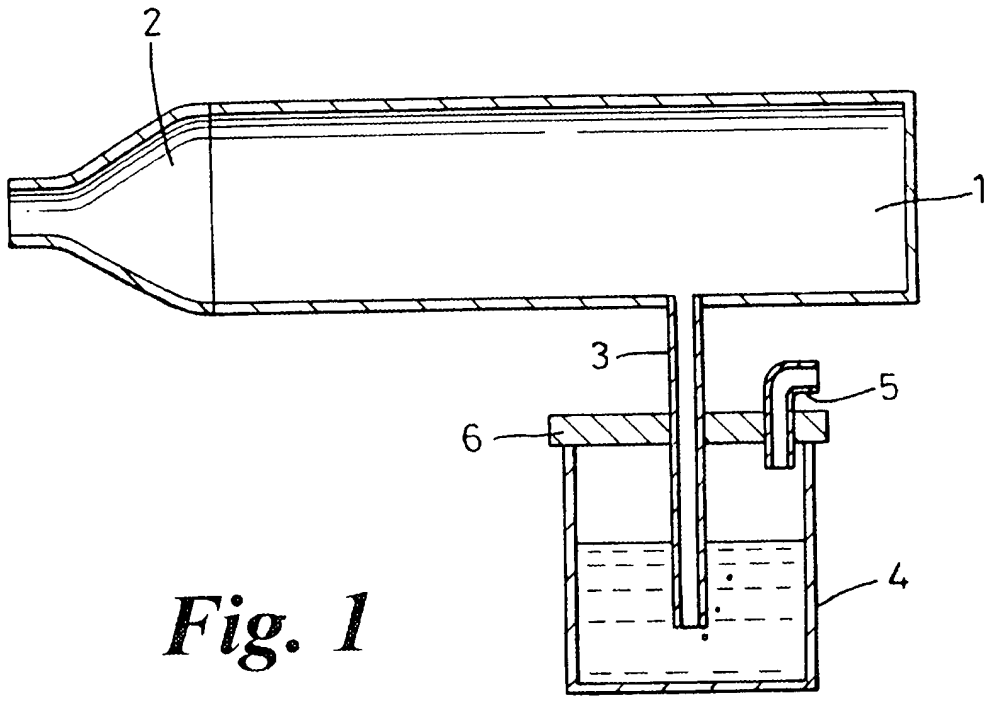

Fig. 1

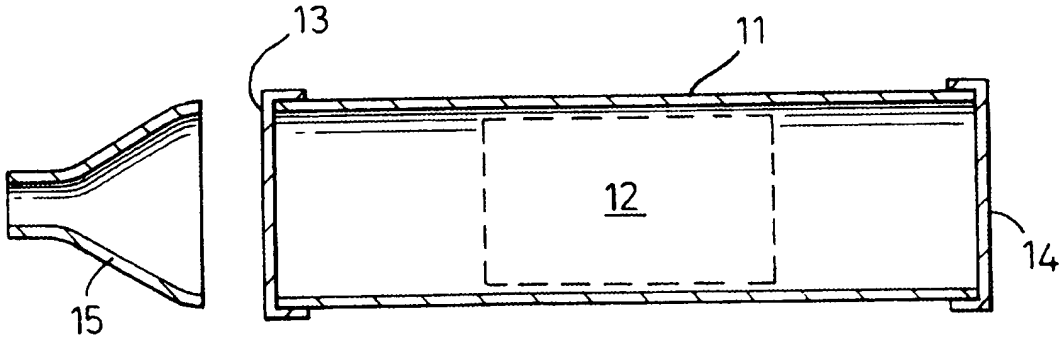

Fig.2

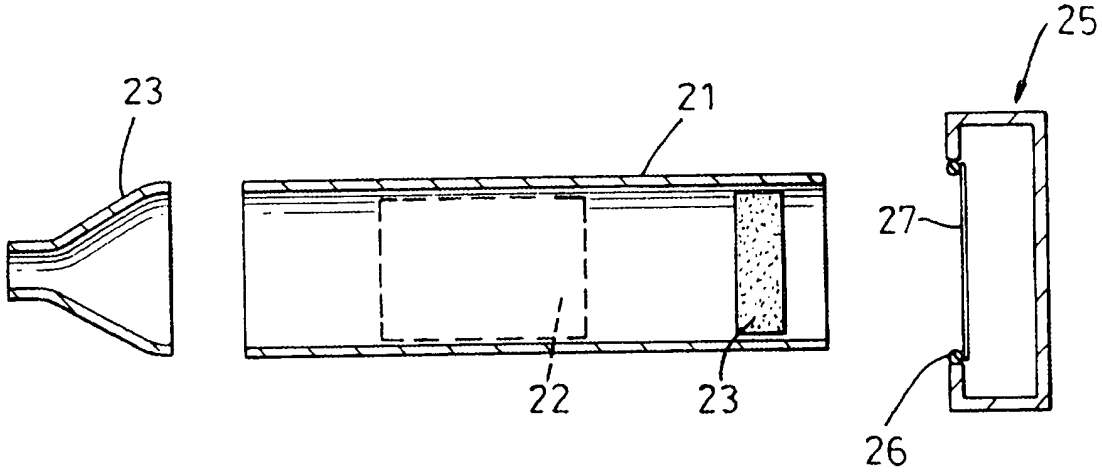

Fig. 3

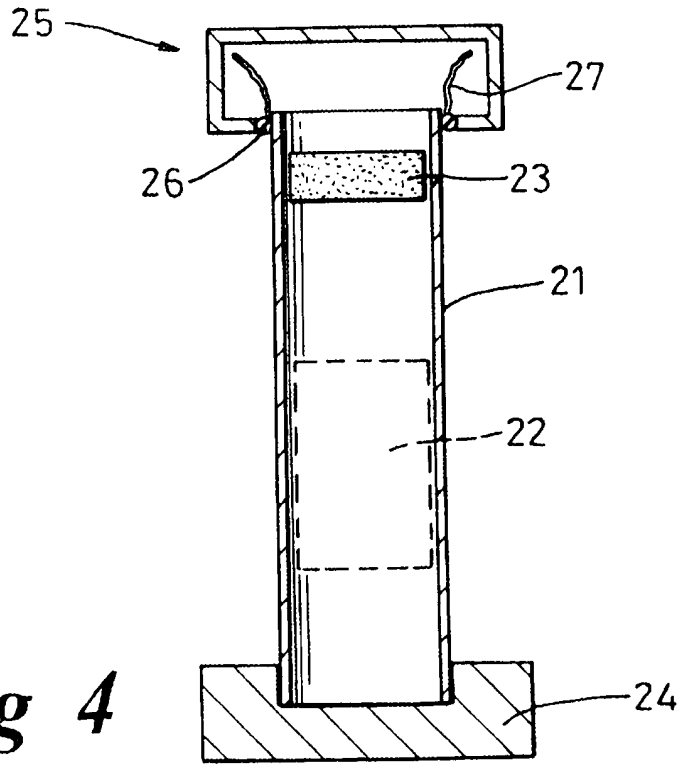

Fig 4

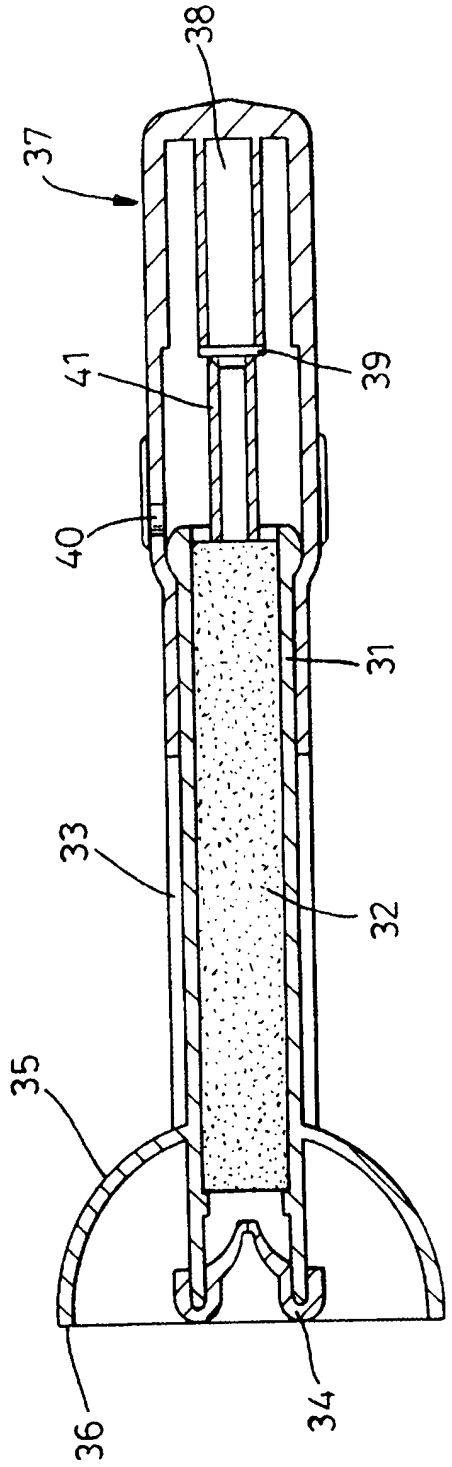

Fig. 5

INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/EP 97/00712

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/497 G01N31/22 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 4 947 861 A (HAMILTON) 14 August 1990 see the whole document ---	1,2,7, 14,15,20 3-6,13, 16-19,26
Y	US 5 364 797 A (OLSON ET AL.) 15 November 1994 see column 7, line 29 - column 8, line 48; claims 1,2,17,33,39; figure 3 ---	3,4,16, 17
Y	WO 91 19192 A (MALLOW, WILLIAM , A. ET AL.) 12 December 1991 see page 4, line 3 - page 10, line 18 --- -/--	5,6,18, 19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 April 1997	10.06.97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 679 721 A (GASTEC CORPORATION) 2 November 1995 see the whole document	1-19
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